Attorney Docket No. 54269.8002.US01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Group Art Unit: Thai-An N. Ton
Kangsheng Wang	Examiner: 1632
Serial No. 09/781,046	
Filed: February 8, 2001	
For: A Method and System for Introducing a Gene into a Human Stem Cell	

AFFIDAVIT OF KANGSHENG WANG

PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

I, Kangsheng Wang, hereby declare, subject to penalty of perjury, as follows:.

1. I am currently President and Chief Executive Officer of BioAgri Corporation ; I have been involved in the research of molecular biology for over 22 years; I obtained a Ph.D. degree in Biology from the California Institute of Technology in 1991; I worked at Chiron Corporation as Research Associate for 3 years and Scientist for 7 years; and I have published about 12 research papers in well known scientific journals.

2. I have reviewed the United States Patent Application No. 09/781,046 entitled "A Method and System for Introducing A Gene Into A Human Stem Cell" filed February 8, 2001 (the '046 Application) and the United States Patent Application No. 09/573,861 entitled "A New Vector

For Genetically Modifying Non-Human Animals" filed March 28, 2000 (the `861 Application), wherein the `046 Application is a continuation-in-part application of the `861 Application.

3. I am the sole inventor of the '046 and '861 Applications.

4. I have read and understood the Office Action dated April 26, 2002 and the Final Office Action dated February 11, 2003, regarding the `046 Application. The Final Office Action maintains the rejections of claims 22 – 26 of the `046 Application under 35 U.S.C.112, first paragraph, since the Final Office Action asserts that "in general antibodies directed to sperm would be expected to inhibit fertilization" and "it would have required undue experimentation for one skilled in the art to expect that the sperm-specific antibodies of the instant invention would retain the ability to fertilize an oocyte."

5. I have made more than one antibodies that bind to a sperm, retain the ability of the sperm bound with the antibody to fertilize an oocyte, and carry DNA into the oocyte from which a transgenic animal developed, through the use of the same method as disclosed in the `046 and `861 Applications.

6. I include a copy of pertinent pages of my original laboratory records dated from March 15, 1999 to February 8, 2001, as shown in Appendix A submitted herein.

Page 4 of the Appendix A demonstrates that mouse sperm cells were collected and used to immunize mice to produce antibodies against the sperm cells. This procedure is identical to what was described in Example I of the `861 Application (p. 10, ll. 20-21, the `861 Application).

Pages 6 to 10 of the Appendix A show that a number of hybridoma supernatants generated from the mice immunized by mice sperm cells do not prohibit sperms from fertilizing oocytes. In particular, hybridoma supernatants were incubated with sperm cells first and the mixture was

incubated with oocytes for *in vitro* fertilization (Page 6). It was observed that hybridoma supernatants nos. 1B3, 1F5, 2D4, 2E8, 3C7, 4E7 did not inhibit fertilization. In a re-testing process, sub-supernatant 1A8 from 1B3, sub-supernatant 1F3 from 2D4, sub-supernatant 2C5 from 3C7, sub-supernatant 2G5 from 2E8, sub-supernatant 1F11 from 4E7, and sub-supernatant 1D8 from 1F5 all retained fertilization (Page 9 of the Appendix A).

Pages 12 to 20 of the Appendix A illustrate that the hybridoma supernatants that retained fertilization contained antibodies that bind to sperm cells. As shown in page 10, hybridoma supernatants 1B3(1A8), 2D4 (1F3), 3C7(2C5), 2E8(2G5), 4E7(1F11), 1F5(1D8) were marked as mouse antibody A, B, C, D, E and F respectively. The flow cytometry method as disclosed in Example I of the `861 Application (p. 10, l. 21 to p. 11, ll. 8, the `861 Application) was conducted to determine whether the antibodies bind to sperm cells. It was observed that mouse antibody A which is mAbA bound to mouse sperm cells (Page 17), so did mouse antibody B which is mAbB(Page 18), mouse antibody C which is mAbC (Page 19), and mouse antibody D which is mAbD (Page 20).

Pages 22 to 23 further illustrate that mAbC and mAbD both have shown to carry transgene DNA into an oocyte from which a transgenic mouse develops. The procedures to generate a transgenic mouse are identical to Example IV of the `861 Application (p. 15, l. 1 to p. 16, l. 6 of the `861 Application). The Southern blot analysis was performed to confirm whether the transgene DNA was integrated into the genome of the transgenic mouse (Example IV of the `861 Application). As shown in page 22 of the Appendix A, the transgenic mice generated using mAbC contained the transgene in their genome. The transgenic mice generated using mAbD also contained the transgene their genome (page 23 of the Appendix A).

In light of the foregoing, it is concluded that a number of antibodies, including 1B3(1A8),

2D4 (1F3), 3C7(2C5), 2E8(2G5), 4E7(1F11), and 1F5(1D8), have been made according to the method disclosed in the '861 Application, and have binding affinity to sperm cells and the sperm cells bound with the antibodies retain the ability to fertilize oocytes. In addition, more than one antibody (mAbC and mAbD) has demonstrated that sperm cells bound with the antibody are able to carry transgene DNA and fertilize an oocyte from which a transgenic animal develops and contains the transgene.

7. I note that the mAbC in the `046 Application is identical to the mAbC in the '861 Application and is made by the method as disclosed in the `861 Application.

8. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and, further, that these statements are made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of United States Applications No. 09/781,046 and United States Patent Application No. 09/573,861, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed and signed on Oct 13, 2003, at City of Industry, California,

Kangsheng Wang

Kangsheng Wang

Linker Based Sperm-Mediated Gene Transfer Technology

1. Over-Immunization of Balb/C Mice with Mouse Sperm Cells

2. Screen Hybridomas Which Does Not Prevent Sperm Fertilization by In Vitro Fertilization

3. Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells

4. Generation of Transgenic Mice from Two Different Linkers mAb C and mAb D

Over-Immunization of Balb/C Mice with Mouse Sperm Cells

Work continued from Page

1. Immunize 3 Balb/c mice with 2×10^6 ~~B6D2F1~~ B6D2F1 and FVB male sperm male sperm mix with Ajuvant overimmune 8 time (twice/month) (2 for FVB sperm) (1 for B6D2F1 sperm)

a. dissect epididymis of 12-15 weeks olds male, squeeze the sperm out ~~from~~ and let sperm in Modified Tyrode's medium without BSA.

b. wash sperm with MTM three time, count Number and ~~immunize~~ mix with 200ul TDM.

c. Immunize 5 weeks old Balb/c female. (twice/month)

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Screen Hybridomas Which Does Not Prevent Fertilization by In Vitro Fertilization

(screening assay shows that a number of hybridomas supernatants does not inhibit sperm cells bound with antibodies in the supernatant to fertilize oocytes.)

Work continued from Page

Test in vitro fertilization efficiency and blocking by mAb (hybridoma supernatant)

1. Set 20 egg/assay for IVF study, ~~and~~

2. inject ~~with~~ 5I.U. PMS (Sigma) ~~to~~ in 8 p.m. (day 1)
50 B6D2F1 female with 8 weeks old

3. 48 hours later, each mouse ~~we~~ was injected with 5I.U hCG (day 3)

4. On day 4 at 7:30 a.m., ~~the~~ Sacrifice the female mice and collect egg ~~from~~ with cumulus cell from swollen ampulla in MTM medium.

5. ~~Add~~ Distribute ~~each~~ one clump of cumulus cell with egg to each ~~w~~ ~~48~~ well of 48 well dish in 200 µl MTM medium (average 20 eggs)

6. Add 20 µl of supernatant of hybridoma to each well and incubate with 5×10^4 sperm in 30 µl MTM medium for 30 min

7. add sperm mix in (6) to (5). and incubate in 37°c for 4 hr for in vitro fertilization

8. ~~collect~~ collect and transfer fertilized ~~100~~ eggs to CZB medium and incubate at 37°C for 20-22 hrs ~~in 96 well~~

9. observe the fertilization efficiency
(+++) No block fertilization ~~very well~~ of sup. of hybrido.
~~(+++)~~
(++) block ~~ok~~ some
(+) block ~~was~~ a little
blank blocked

Work continued to Page

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group 1 (+++ or ++)

1 B3
1 F5
2 D4 (6)
2 E8
3 C7
4 E7

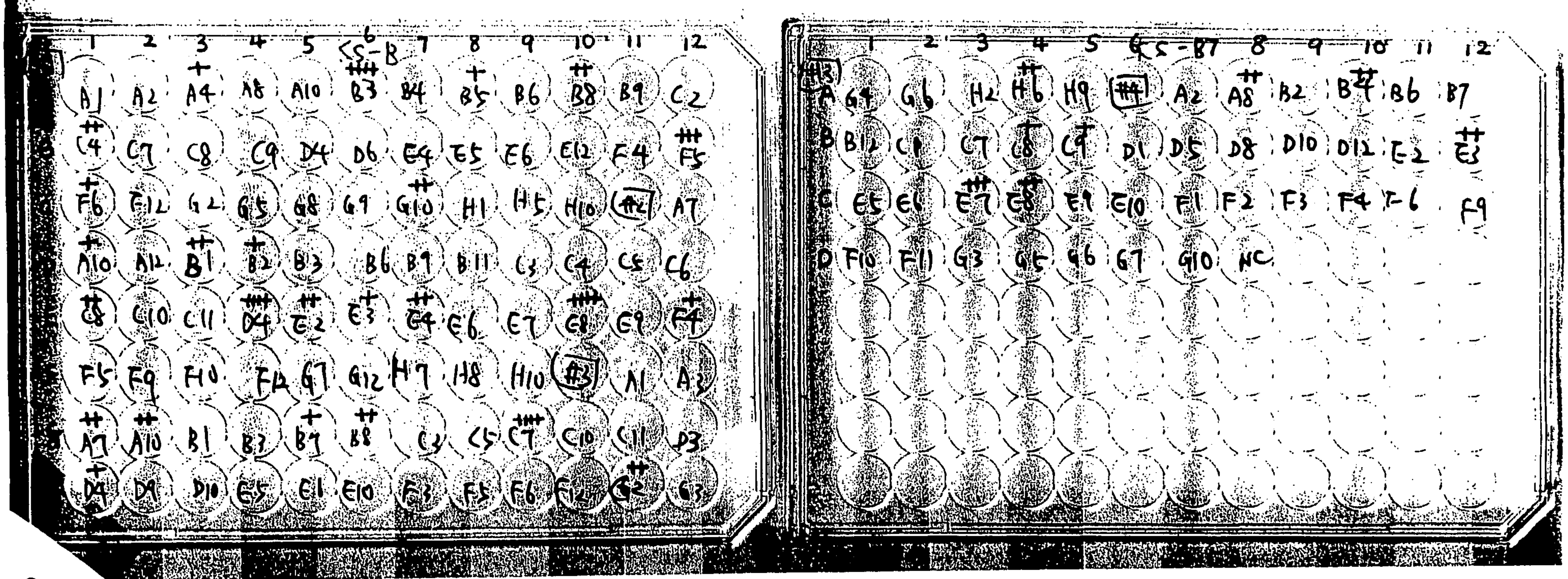
group 2 (++)

1 B8
1 C4
1 G10
2 B1
2 C8
2 E2
2 E4
3 A7
3 A10
3 B8
3 G2
3H6
4A8
4B4
4E3
4E8
(16)

group 3 (+)

1 A4
1 B5
1 F6
2 A10
2 B2
2 E3 (11)
2 F4
3 B7
3 D4
4 C8
4 C9

Supernatant from hybridoma from ~~mouse~~ Bulb/c V immunized with FVB and B6D2F1 mouse sperm



group 1 (++++ or +++)

1A7
1C5
1C12
1F3
1H12
2B10
2D5
2D8
2E10
3A6
4C10

group 2 (++)

1A5
2A1
2A2
3A5
3C6
3C8
3D7
3E9
3G8
4E11

group 3 (+)

1C1
1C6
1D9
2A11
2B4
2B7
2B7
3C4
4E12
4G4

Retest

~~Two time~~

① show (++++ or +++) ~~two time~~ twice

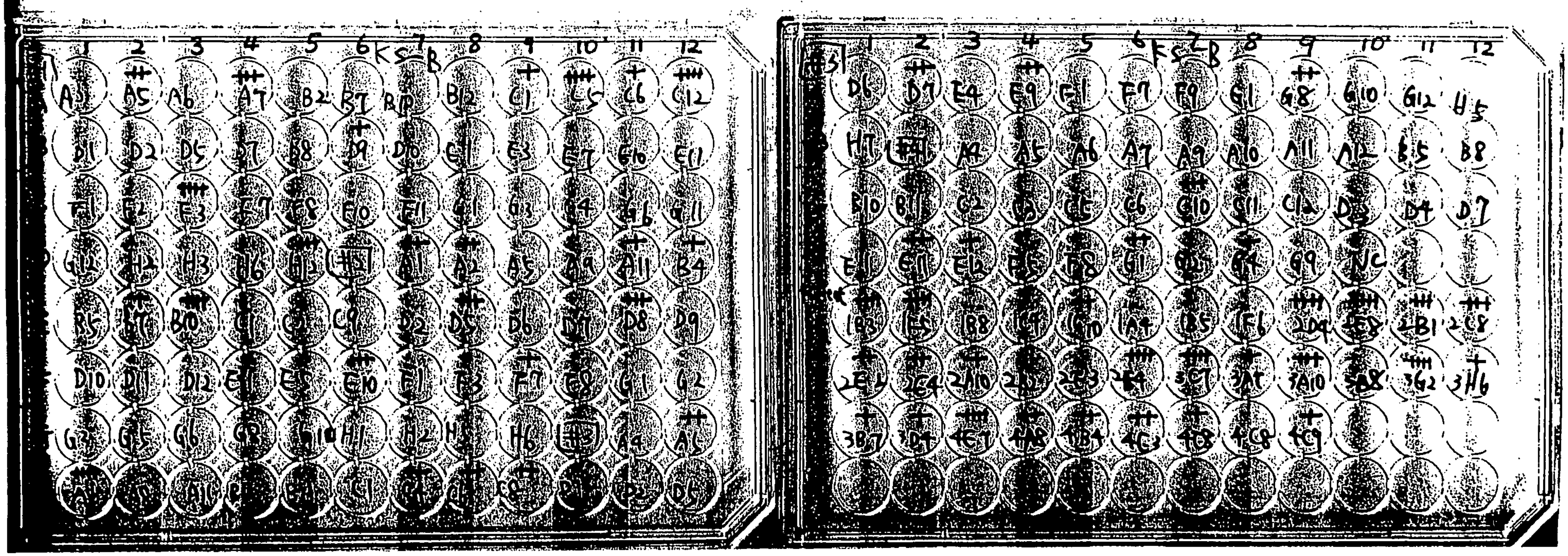
1B3
1F5
2D4
2E8
3C7
4E7

② show +++ once ++ once

2B1
2C8
3A10
3G2

③ show ++ twice

1G10
2E2
2E4
4A8
4B4
4E3



B subclone

1B3

- △ 1A8 (+++) twice
- 2A2 (+++) twice
- 2E7 (+++) twice

2D4

- △ 1F3 (+++) once (+) once
- 1G4 (+++) once (+) once
- ✓ 1G2 (++) once
- ✓ 1G9 (++) once
- ✓ 2B8 (++) once
- ✓ 2C3 (++) once

3C7

- △ 2C5 (++) twice
- 1D8 (++) once (+) once
- 2C6 (++) once (+) once
- 2D10 (++) once (+) once

2E8

- △ 2G5 (+++) twice
- 2G8 (+++) twice
- 1C10 (++) twice

4E7

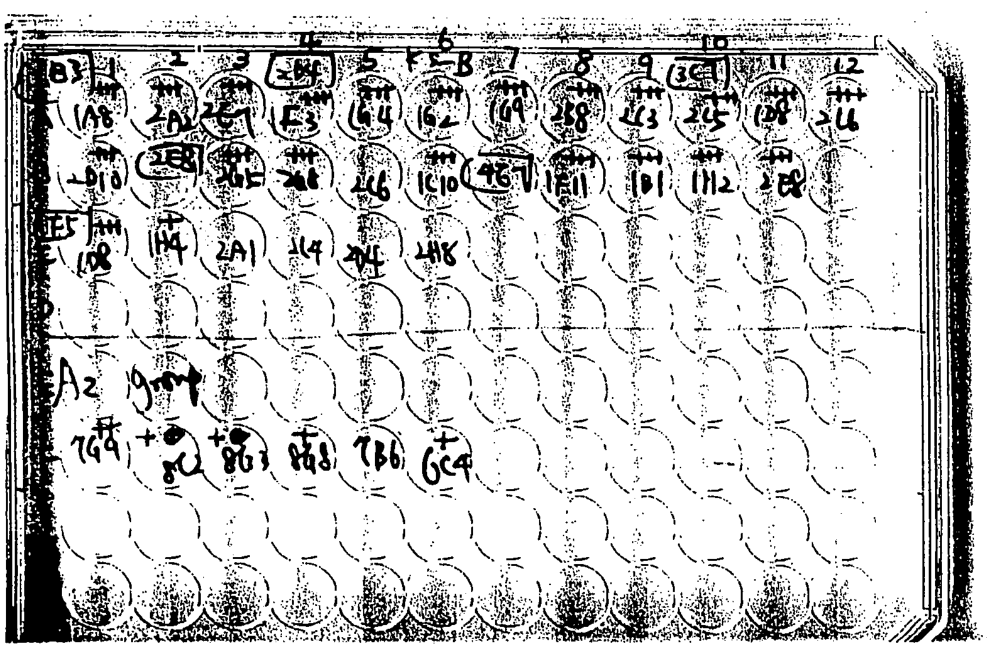
- △ 1F11 (+++) twice
- 1B1 (++) twice
- 1H2 (++) twice
- 2E8 (++) twice

1F5

- △ 1D8 (++) once (+) once
- ✓ 1H4 (+) once

A2 group

- △ 7G9 (++) once (+) once
- △ 8C2 (+) twice
- △ 8G3 (+) twice
- △ 8G8 (+++) once (+) once (0) once
- △ 6C4 (+++) once (+) ~~once~~ twice
- 7B6 (+) twice (0) once



TITLE

PROJECT NO.

BOOK NO.

B group do isotype assay, we got all IgM

so we select $\boxed{1B3}$ 1A8 (A) to do ascite fluid

$\boxed{2D4}$ 1F3 (B)

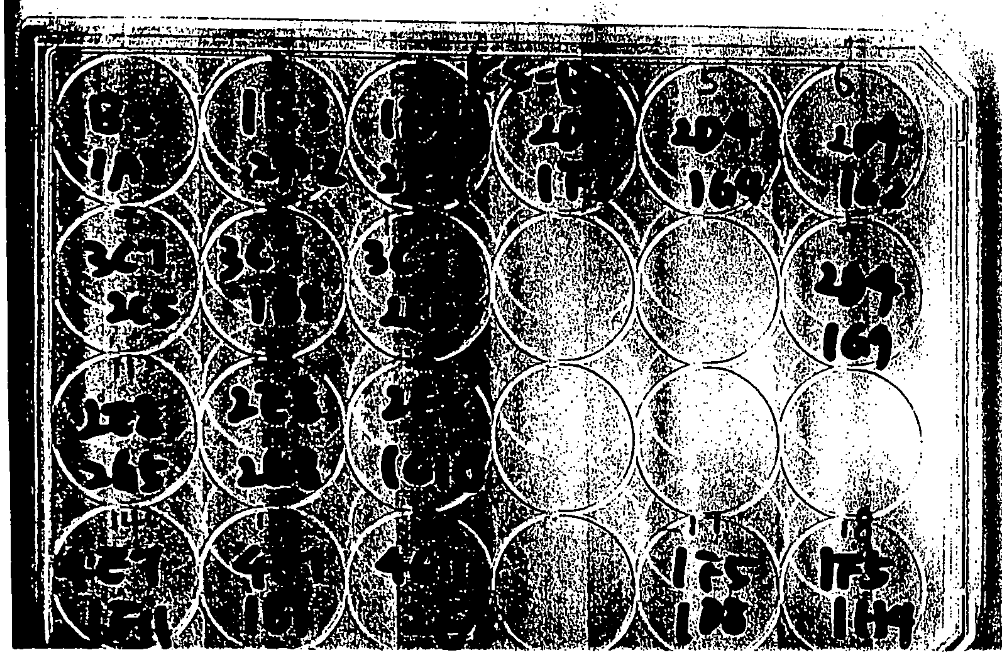
$\boxed{3C7}$ 2C5 (C)

$\boxed{2E8}$ 2G5 (D)

$\boxed{4E7}$ 1F11 (E)

$\boxed{1F5}$ 1D8 (F)

✓



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SIGNATURE K. Wang

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DATE

WITNESS

DATE 10-1-99

Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells

(Four mAbs A, B, C and D show the binding of mouse sperm cells)

9/2

COW

BAG (Ken)
FITC

2 Sep 99
1530-1645

HeNe
488(I70)/500mW

atter	L1	P1	Log	PM1	N. ~~BOND~~
90	L1	P2	Log	580V	PMT 1
ITC	L2	P3	Log	620V	3
ute	L2	P4	Log	550V	5

No Threshold

60.5/60.0

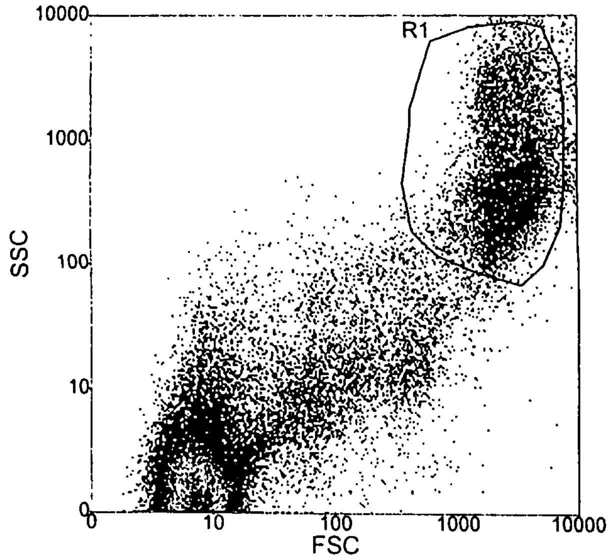
BAG-1999-09-02-000 Bovine Extender Auto

Bovine Sperm #4

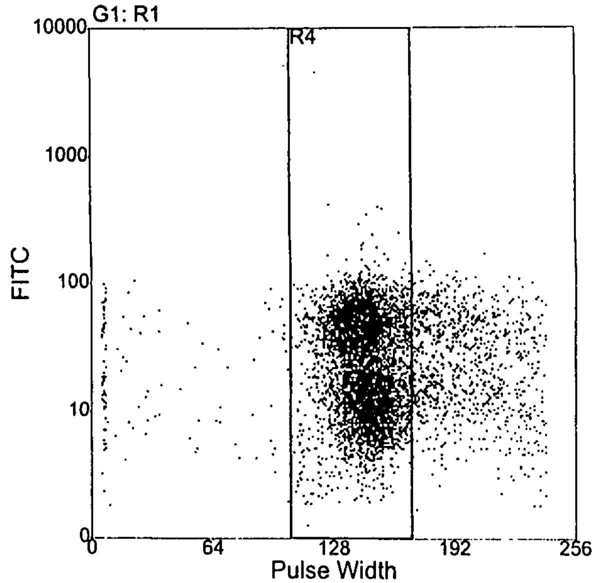
-001	Bovine	Extender	2nd only
-002	"	"	Myelin
-003	"	"	C
-004	"	"	D
-005	"	PBS	Auto
-006	"	"	2nd only
-007	"	"	Myelin
-008	"	"	C
-009	"	"	D

-010	Mouse	Auto
-011	"	2nd only
-012	"	~~Myelin~~ Tubulin
-013	"	Myelin
-014	"	A
-015	"	B
-016	"	C
-017	"	D

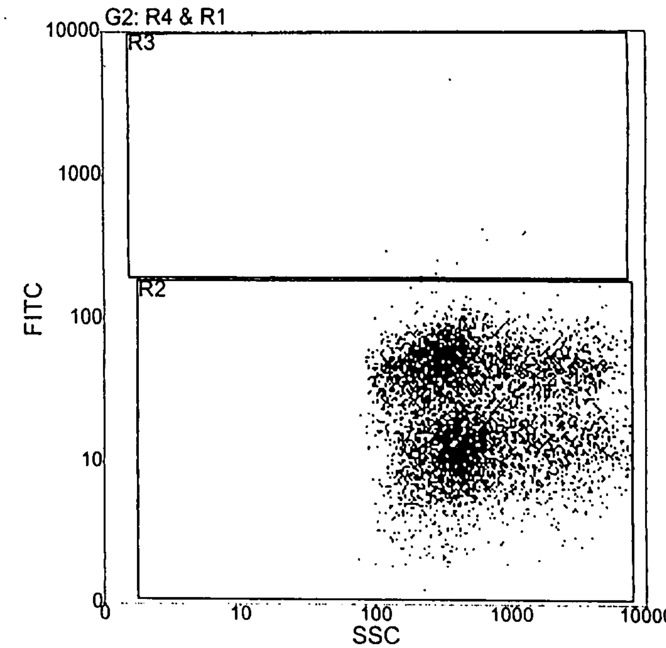
Mouse Auto Mouse Sperm #4 E:\Results\BAG-1999-09-02-010.fcs



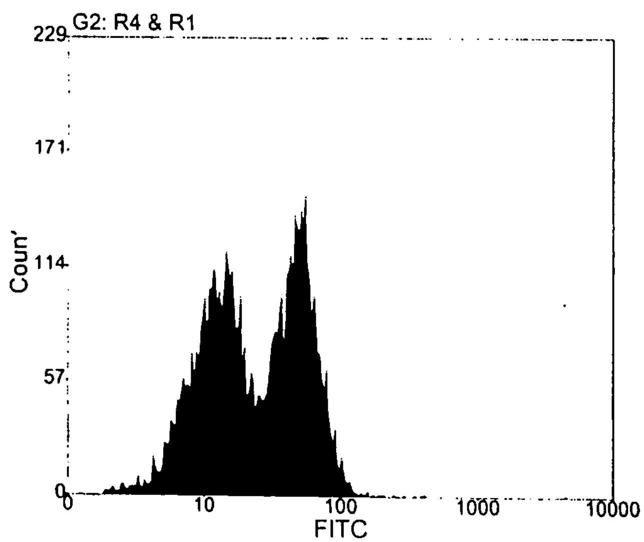
Region	Count	%	Mean
Total	50000	100.00	598.87, 192.30
R1	8385	16.77	2853.56, 898.98



Region	Count	%	Mean
Total	8385	100.00	155.64, 32.50
R4	6764	80.67	143.52, 32.12

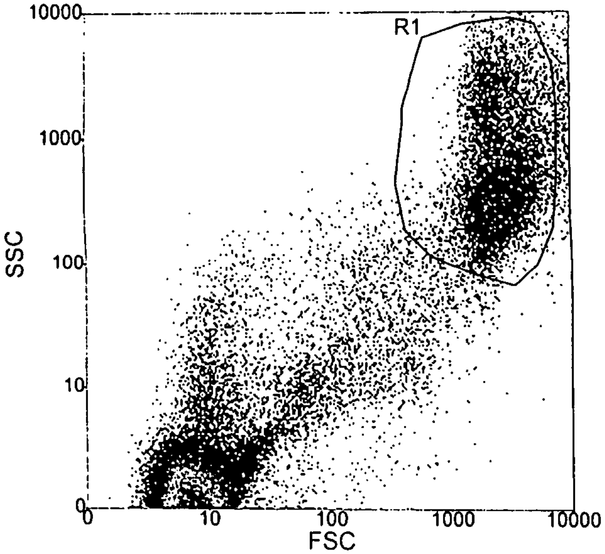


Region	Count	%	Mean
Total	6764	100.00	886.10, 32.12
R2	6753	99.84	886.72, 31.10
R3	11	0.16	507.41, 659.13

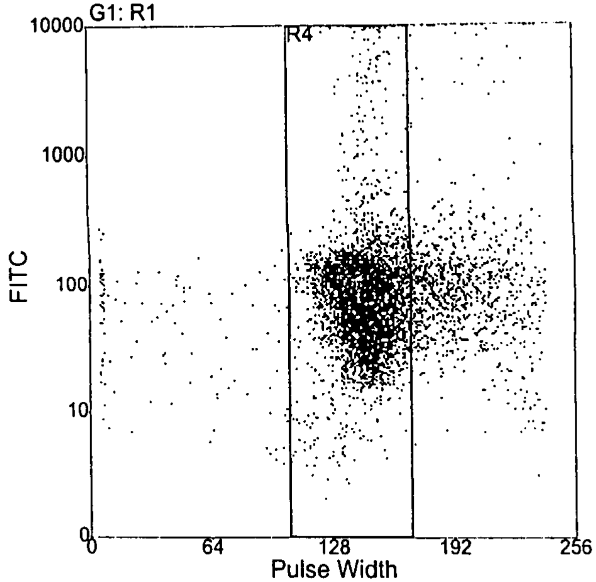


Region	Count	%	Mean
Total	6764	100.00	32.12

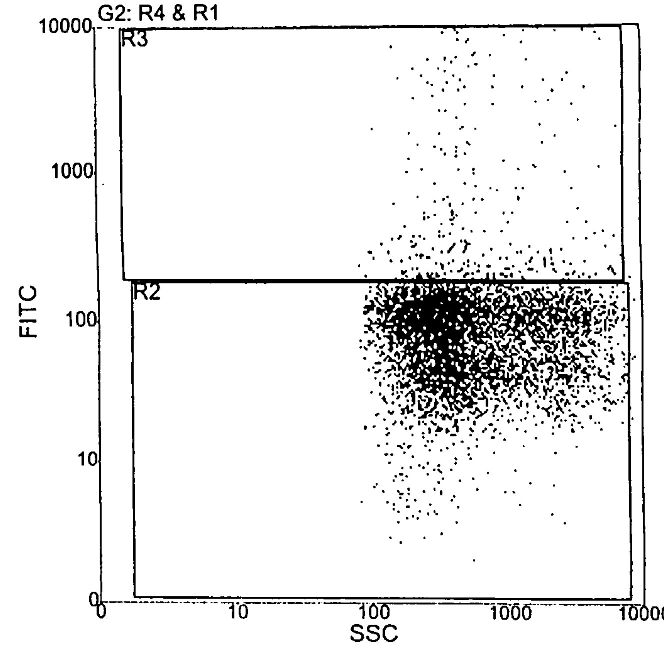
Mouse 2nd only   Mouse Sperm #4   E:\Results\BAG-1999-09-02-011.fcs



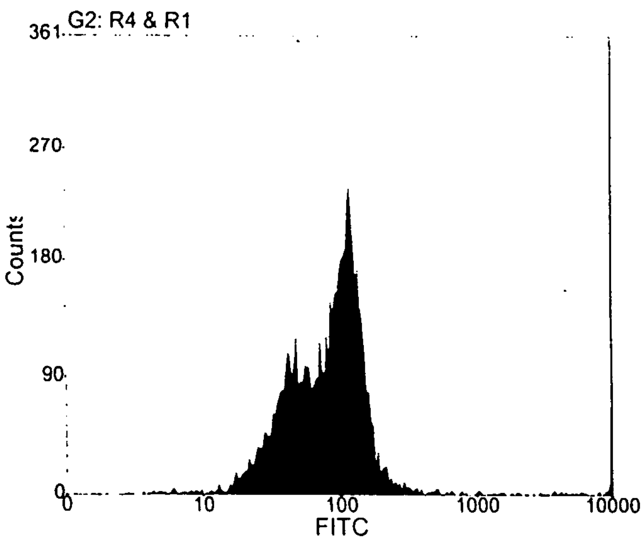
Region	Count	%	Mean
Total	50000	100.00	619.91, 204.17
R1	8714	17.43	2700.21, 895.86



Region	Count	%	Mean
Total	8744	100.00	156.37, 300.47
R4	6912	79.05	142.58, 283.17



Region	Count	%	Mean
Total	6912	100.00	875.93, 283.17
R2	6464	93.52	863.97, 83.17
R3	349	5.05	1063.07, 1468.16

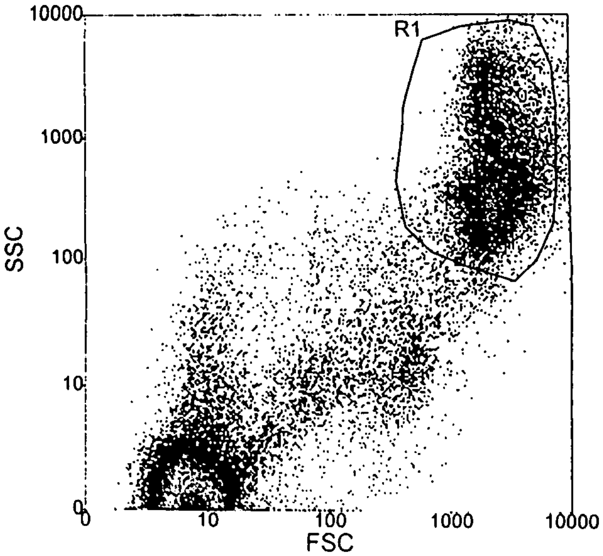


Region	Count	%	Mean
Total	6912	100.00	283.17

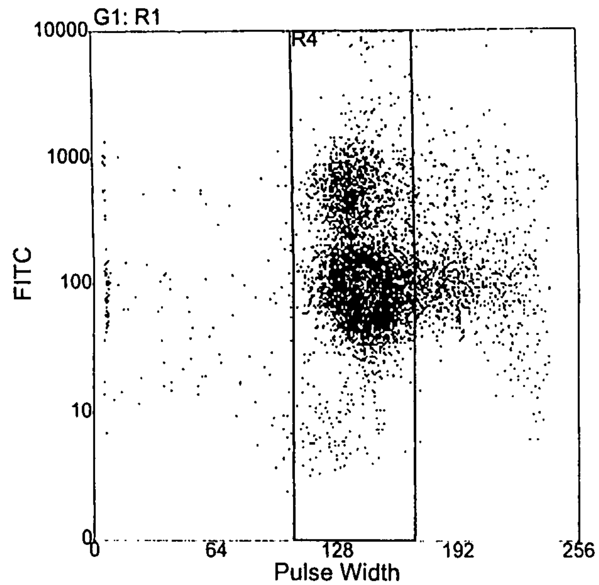
Mouse Tublin

Mouse Sperm #4

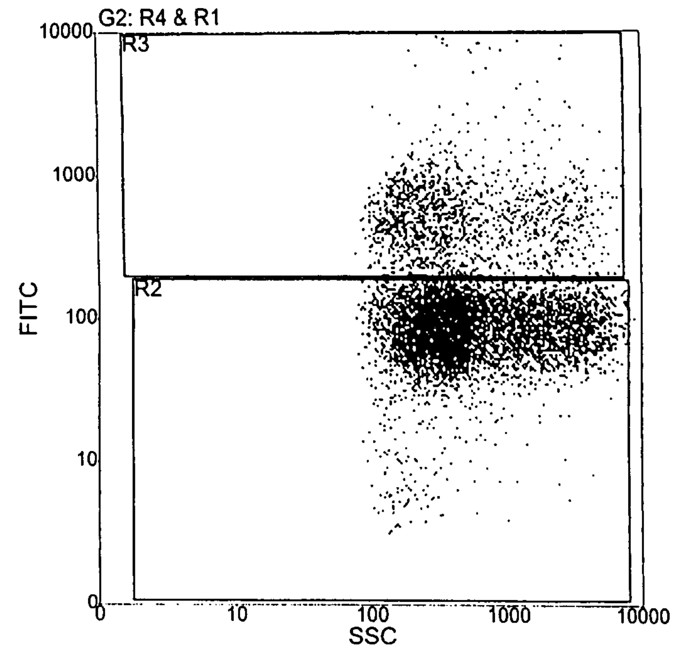
E:\Results\BAG-1999-09-02-012.fcs



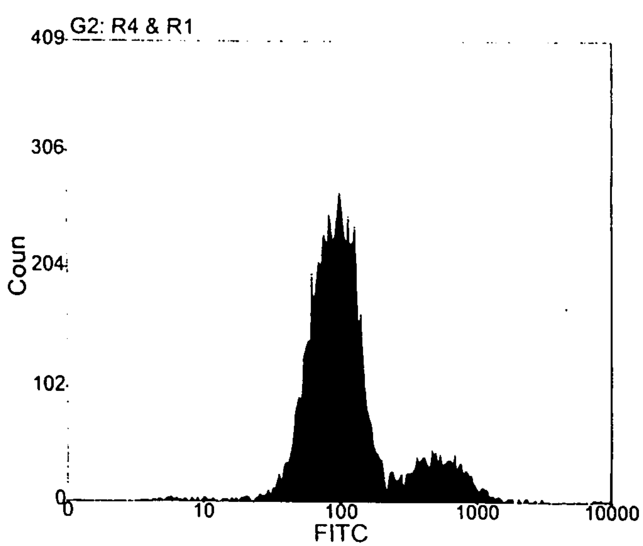
Region	Count	%	Mean
Total	50000	100.00	653.81, 221.67
R1	10891	21.78	2486.54, 886.74



Region	Count	%	Mean
Total	10920	100.00	151.42, 247.95
R4	9111	83.43	140.98, 226.57



Region	Count	%	Mean
Total	9111	100.00	869.49, 226.57
R2	7448	81.75	876.97, 90.06
R3	1627	17.86	837.18, 648.93

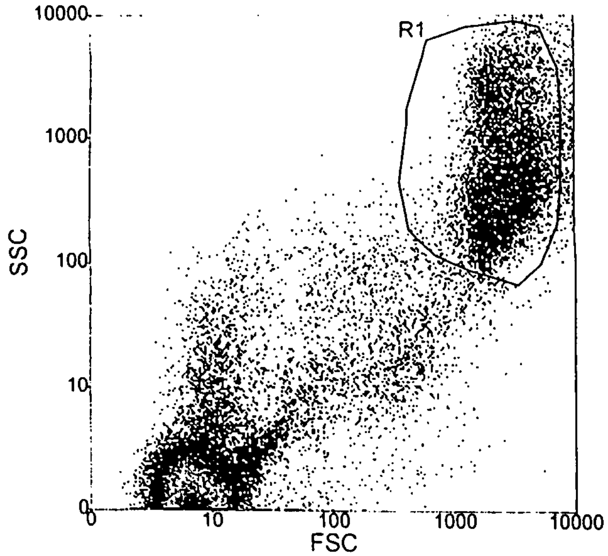


Region	Count	%	Mean
Total	9111	100.00	226.57

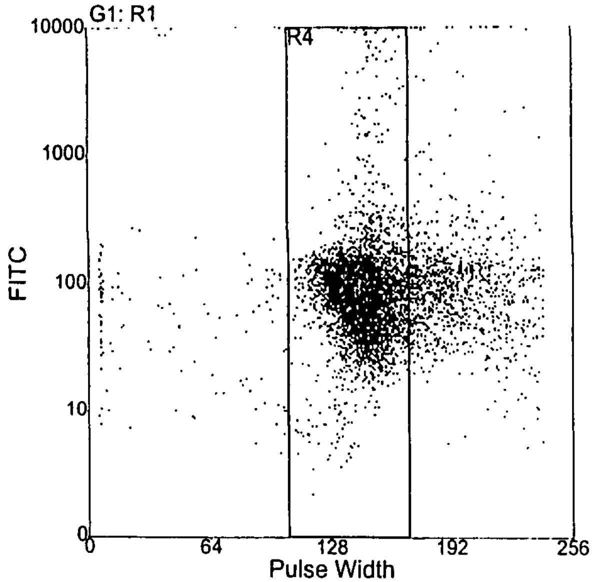
Mouse Myelin

Mouse Sperm #4

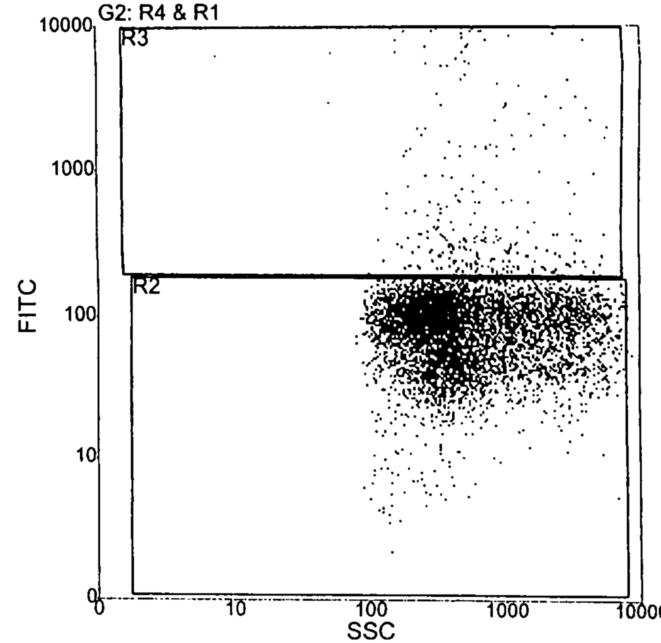
E:\Results\BAG-1999-09-02-013.fcs



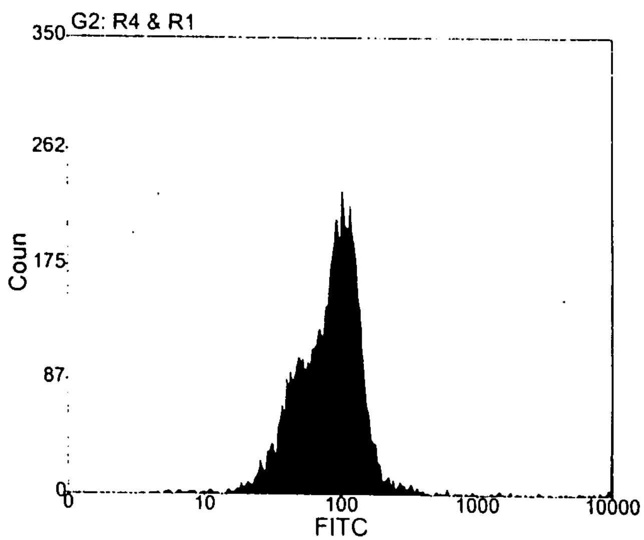
Region	Count	%	Mean
Total	50000	100.00	619.08, 197.16
R1	8660	17.32	2737.25, 902.77



Region	Count	%	Mean
Total	8695	100.00	157.27, 278.34
R4	6802	78.23	142.89, 245.51

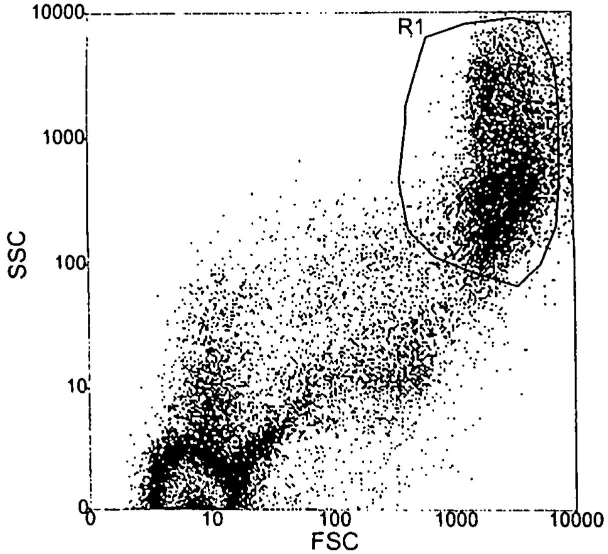


Region	Count	%	Mean
Total	6802	100.00	870.77, 245.51
R2	6438	94.65	860.34, 85.49
R3	286	4.20	1055.34, 1317.32

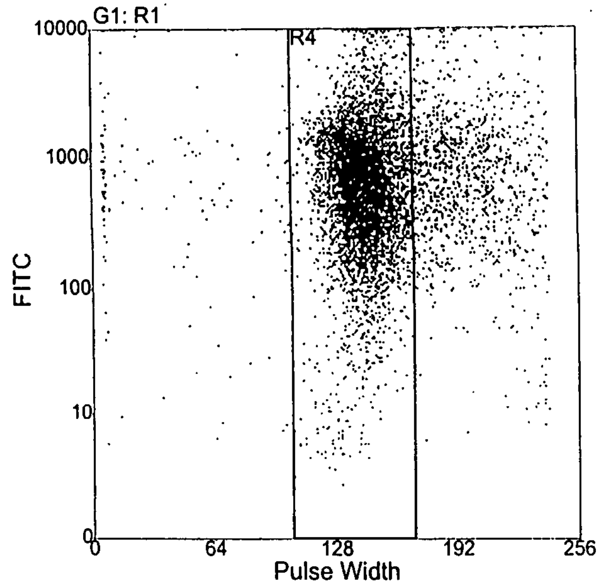


Region	Count	%	Mean
Total	6802	100.00	245.51

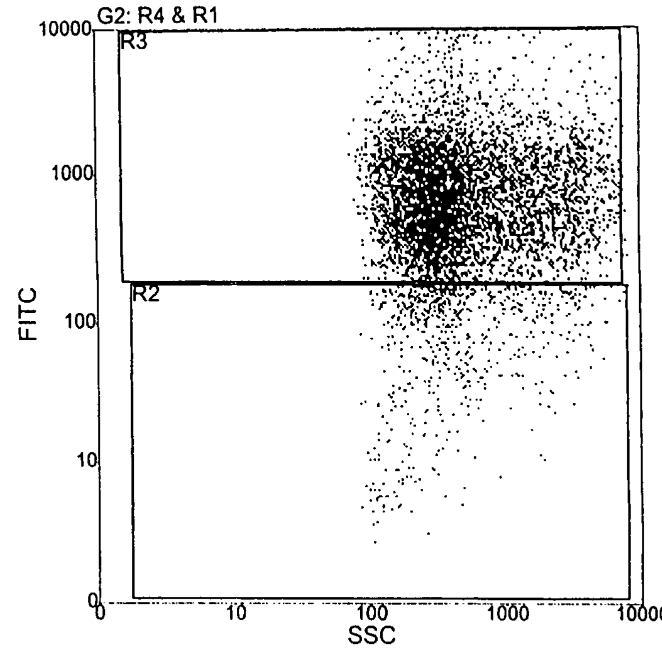
Mouse A Mouse Sperm #4 E:\Results\BAG-1999-09-02-014.fcs



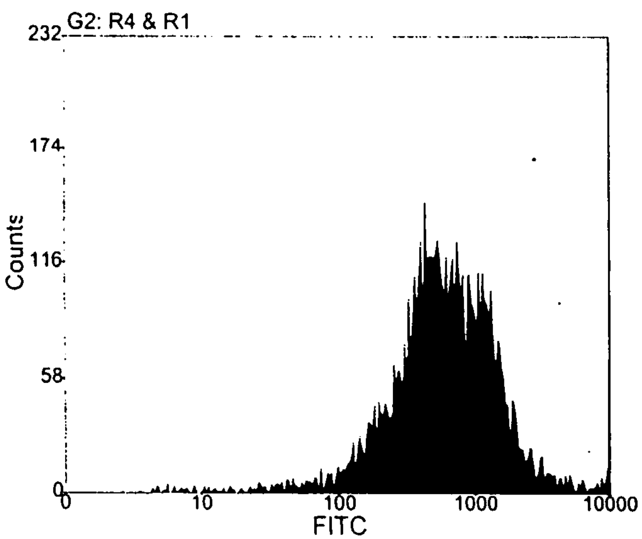
Region	Count	%	Mean
Total	50000	100.00	616.58, 195.81
R1	8966	17.93	2723.48, 879.04



Region	Count	%	Mean
Total	8994	100.00	156.57, 1082.71
R4	7083	78.75	142.66, 994.57

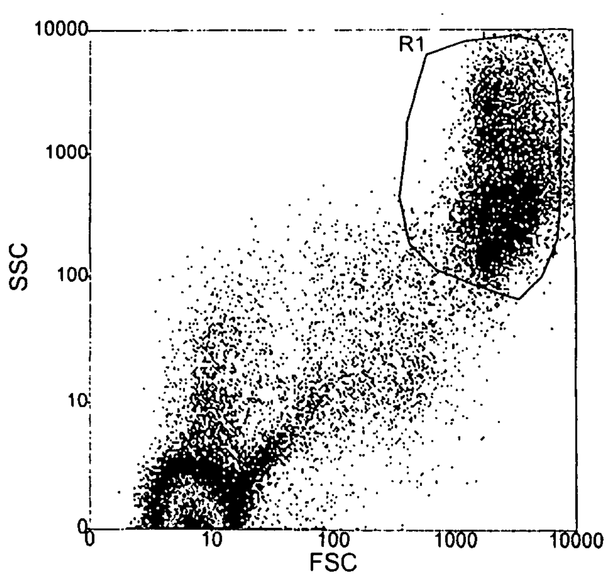


Region	Count	%	Mean
Total	7083	100.00	845.48, 994.57
R2	759	10.72	764.37, 104.30
R3	6211	87.69	852.70, 950.42

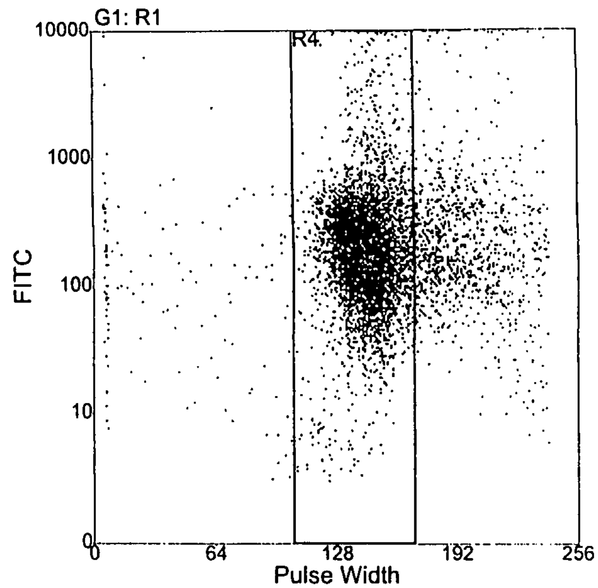


Region	Count	%	Mean
Total	7083	100.00	994.57

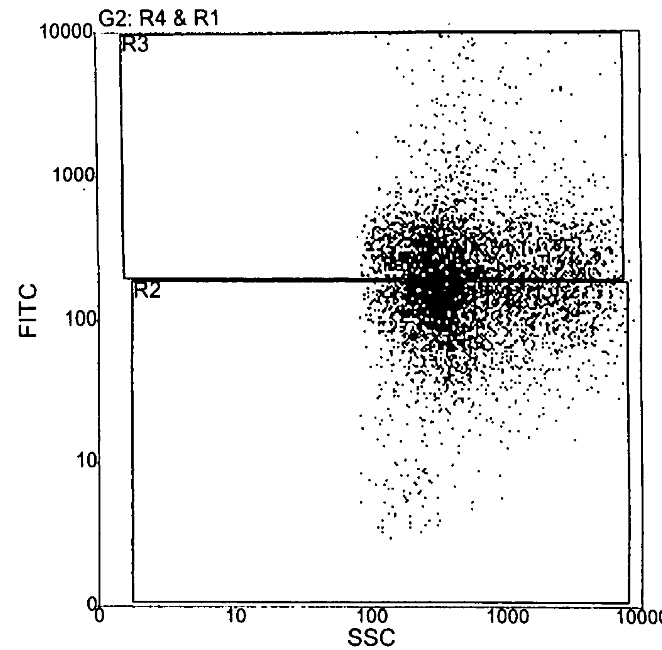
Mouse B Mouse Sperm #4 E:\Results\BAG-1999-09-02-015.fcs



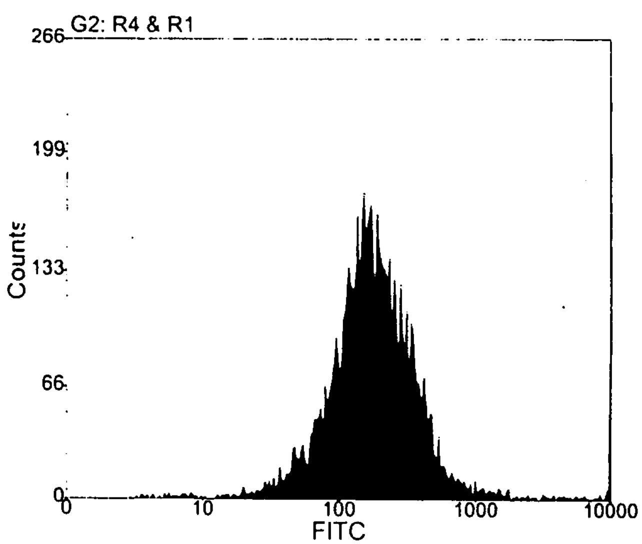
Region	Count	%	Mean
Total	50000	100.00	614.11, 202.03
R1	8893	17.79	2725.28, 886.85



Region	Count	%	Mean
Total	8915	100.00	155.73, 468.50
R4	7091	79.54	142.51, 407.76

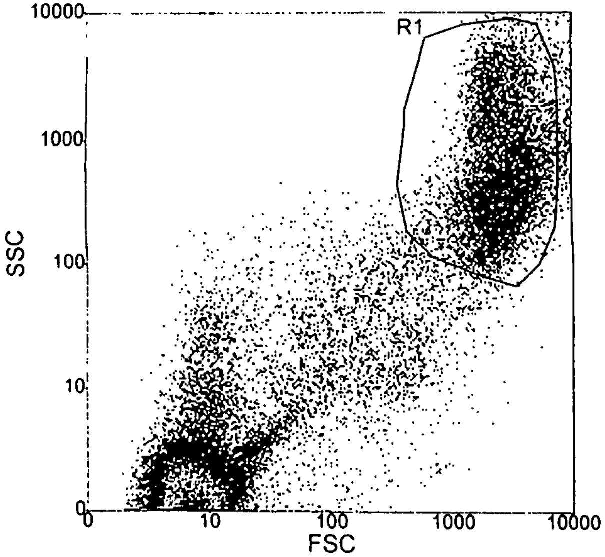


Region	Count	%	Mean
Total	7091	100.00	844.39, 407.76
R2	3930	55.42	809.01, 114.17
R3	3062	43.18	881.24, 497.85

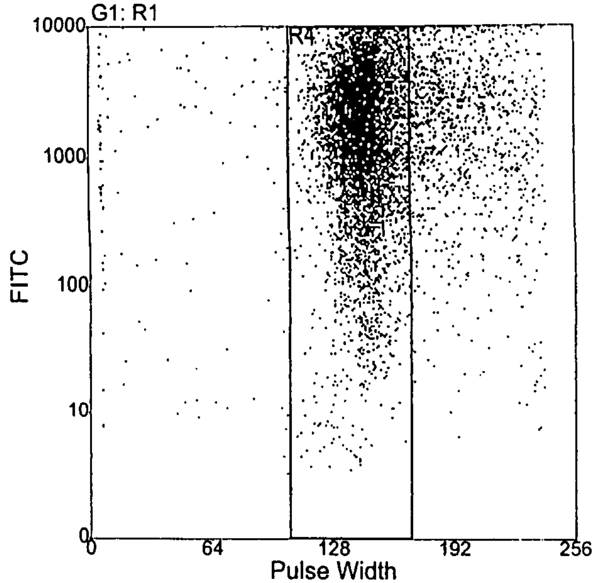


Region	Count	%	Mean
Total	7091	100.00	407.76

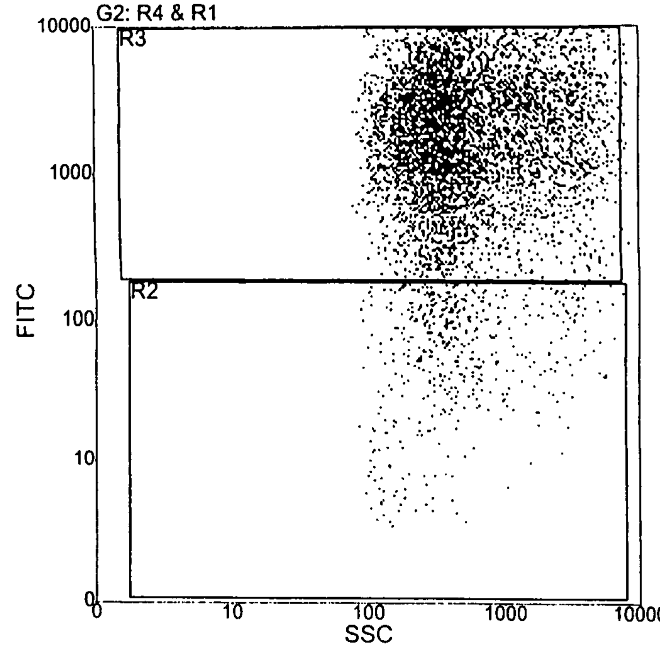
Mouse C   Mouse Sperm #4   E:\Results\BAG-1999-09-02-016.fcs



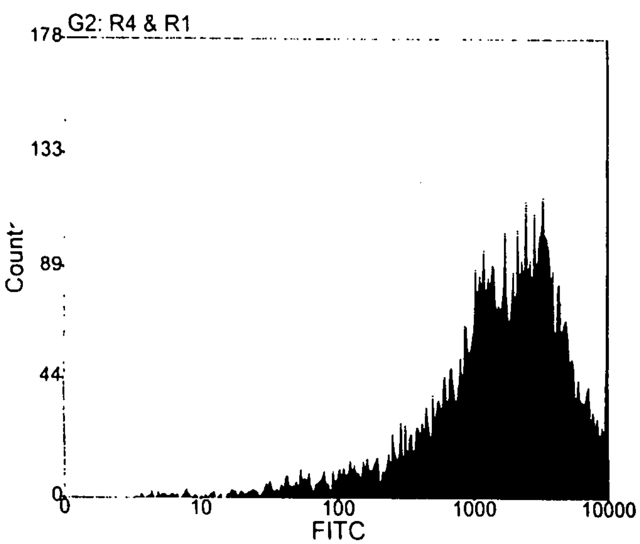
Region	Count	%	Mean
Total	50000	100.00	611.90, 201.63
R1	8939	17.88	2737.66, 905.07



Region	Count	%	Mean
Total	8970	100.00	157.07, 2822.53
R4	7007	78.12	142.94, 2687.92

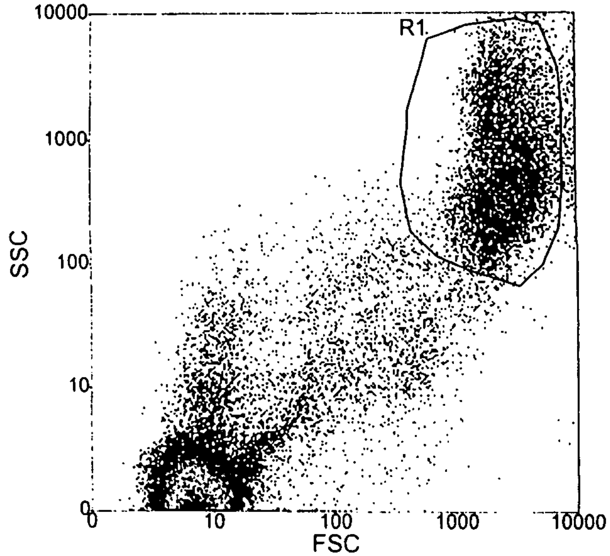


Region	Count	%	Mean
Total	7007	100.00	879.12, 2687.92
R2	529	7.55	824.18, 82.87
R3	6083	86.81	881.79, 2474.10

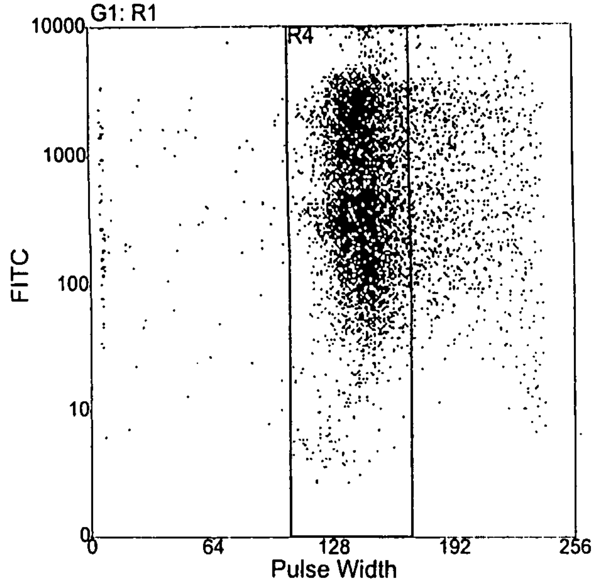


Region	Count	%	Mean
Total	7007	100.00	2687.92

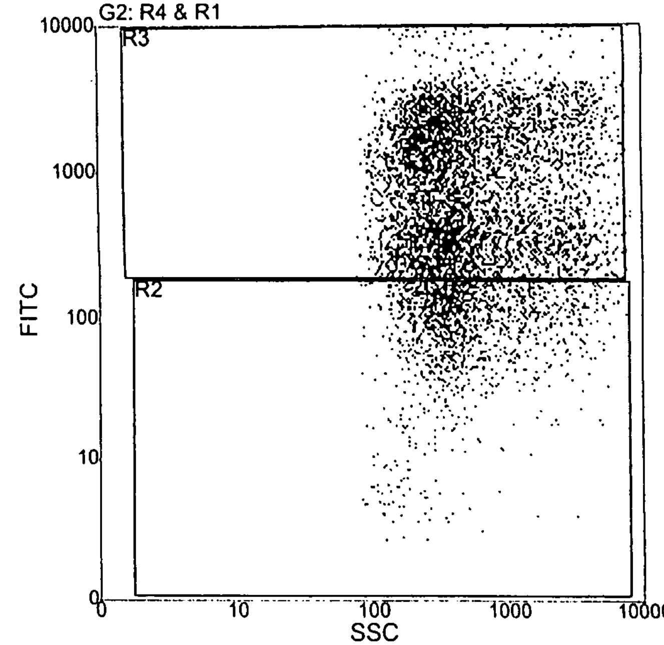
Mouse D   Mouse Sperm #4   E:\Results\BAG-1999-09-02-017.fcs



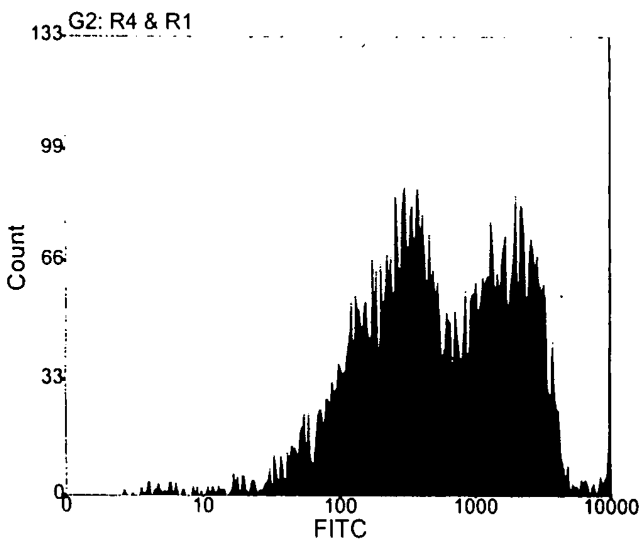
Region	Count	%	Mean
Total	50000	100.00	609.38, 197.20
R1	9020	18.04	2716.36, 880.21



Region	Count	%	Mean
Total	9050	100.00	155.80, 1212.35
R4	7213	79.70	142.79, 1156.87



Region	Count	%	Mean
Total	7213	100.00	867.66, 1156.87
R2	1636	22.68	790.21, 103.34
R3	5467	75.79	888.59, 1304.66

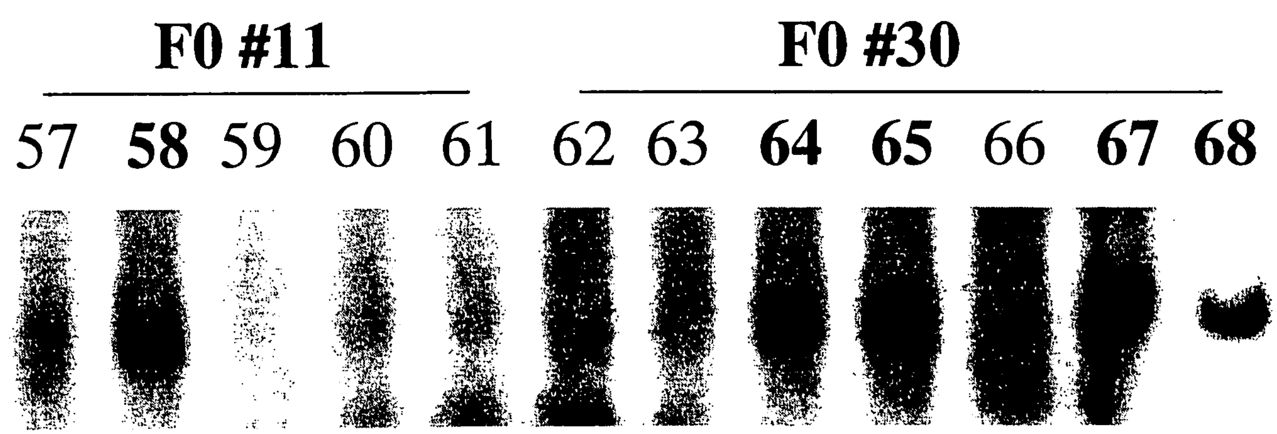


Region	Count	%	Mean
Total	7213	100.00	1156.87

Generation of Transgenic Mice from Two Different Linkers mAb C and mAb D

Transgenic Mice Generated from mAb D Linker

by Southern Blot Analyses



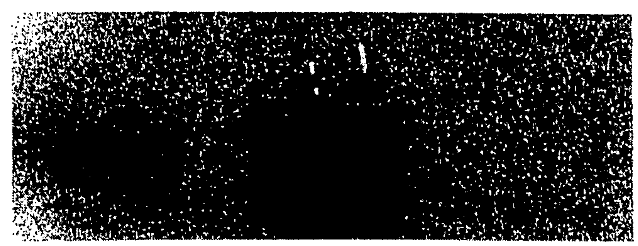
Date: November 17, 2000

Transgenic Mice Generated from mAb C linker

by Southern Blot Analyses

F0 #46

30 32 33



Date: February 8, 2001